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13. ABSTRACT ***(Maximum 200 Words)***

The TACC1 gene was isolated from a region of chromosome 8p11 which shows amplification in breast cancers, especially those which show metastasis to the lymph nodes. Preliminary studies showed that overexpression of TACC1 in mouse fibroblasts cause morphological transformation. We have now shown, using immunohistochemistry and immunoprecipitation, that TACC1 is present in the cytoplasm and the nucleus and furthermore that it probably associates with the mitotic spindle microtubules. Yeast two hybrid analysis has identified a series of genes which interact with TACC1. Two of these, SIAH1 and GAS41, appear to be associated with growth regulatory pathways which may implicate TACC1 in cell growth control and hence why overexpression leads to progression of breast tumors. Two other proteins, p16 and L-Sm7, also interact with TACC1 and these proteins are apparently involved in the transportation of proteins to the nucleus. Thus it appears that TACC1 Makes important associations in the cytoplasm which then facilitate the transportation of TACC1 and probably the whole protein complex into the nucleus where it may affect mitosis. Further characterization of the intracellular pathways which use TACC1 for their function will help further understand its role in breast tumorigenesis.

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Introduction

This is the second year report for this grant which essentially covers a 4 month period from July/00 – October/00. In November/00 the PI relocated his laboratory from the Cleveland Clinic to Roswell Park Cancer Institute. Although the change of grantee institution application has been filed, at the time of writing this report the transfer has not been completed. With delays in providing relinquishing notices from the CCF and delays in effecting the transfer by DOD we have been unable to assign a replacement scientist to the project.

Body

Using computer prediction programs we designed peptides predicted to be antigenic. These peptides were used to raise polyclonal antibodies for TACC1 which are very specific. Using these antibodies we have been able to demonstrate the presence of the TACC1 protein in cell lines available to us. None of the cells lines showed overexpression of TACC1 in these assays. This was unfortunate but, since only 15% of tumors show genomic amplification of the 8p11 region this is not unexpected. It is also possible that any amplification that might have been maintained in tumors could be lost in vitro. The results confirm that TACC1 is expressed in breast cancer cell lines. We are currently extending this analysis to more tumors and normal cells. The only normal cells available to us are MCF10A cells which are hard to propagate and have undergone cytogenetic changes but are generally considered to be 'normal'. The preparation of an antibody and the demonstration of expression of TACC1 in breast tumors cells completes task 1 of the original proposal.

It has been repeatedly demonstrated that DNA amplification results in the overexpression of critical gene(s) within the amplicon, which then results in the increased proliferation of the cancer cell. Southern blot analysis has now suggested that amplification of 8p11 in breast cancer is associated with rearrangement of the 3' untranslated region (3' UTR) of the *TACC1* gene. We would predict that, as has been noted for cyclin D1, the disruption of the 3' UTR could alter the stability of the TACC1 RNA in these tumors. In our initial assessment of the oncogenic potential of TACC1, we used a construct lacking the majority of the 3' UTR. To determine whether the RNA generated from this construct is more stable than the normal TACC1 RNA, we have now constructed the whole 7kb TACC1 construct, containing the whole of the 3' UTR. We have now determined the conditions required to introduce TACC1 constructs into breast tumor cell lines, such as MCF7 and MDA-468, lacking the 8p11 amplicon. We are now in the process of introducing these new constructs into these cells and will generated stable cell lines expressing either the original cDNA, or the full length TACC1. These cell lines will allow us to measure alterations in cellular growth and division rates, to see if either of these constructs lead to a phenotype which mimics the progression to higher grade malignant tumors. Because there is a correlation between 8p11 amplification and

metastasis to the axillary lymph nodes, it will also be important to demonstrate whether these constructs will alter the motility of these cells. We will next perform cell migration assays, to show whether breast cancer cell lines expressing these constructs have an increased ability to migrate *in vitro* in a manner analogous to metastasis, and whether there is a correlation between phenotype and the levels of expression of the TACC1 protein. These overexpressing cell lines will also be used to analyze the intereactions between TACC1 and potential binding proteins in the context of breast cancer cells.

Characterizing the function of TACC1 ultimately depends on establishing its intracellular location. According to PSORT predictions, the predicted 88kD TACC1 protein contains two nuclear localization signals, NLS1 and NLS2. This suggested, but did not prove that TACC1 is localized to the nucleus. We have now determined the normal subcellular distribution of the human TACC proteins using EGFP-tagged proteins and by standard immunohistochemical stains using antibodies generated to the TACC proteins (Gergley et al 2000). During interphase, the TACC proteins are distributed both in the cytoplasm and the nucleus, although antibodies raised to TACC2 strongly stain the centrosomes. In mitotic HeLa and primary fibroblasts, the TACC proteins stain the mitotic spindle and the centrosomes to varying extents. Transient overexpression of the TACC proteins results in the formation of large polymers in the cytoplasm, which retain the ability to bind to microtubules in a regulated manner. This accumulation does not occur in the absence of the TACC domain, suggesting that the TACC proteins interact with microtubules either directly or indirectly through the conserved TACC coiled coil domain. However it is unclear whether the main role of the TACC proteins is in the organization of the microtubule network, or that TACC proteins perform additional functions distinct from their association with microtubules.

To elucidate the potential function of TACC 1, we have chosen to identify proteins which may interact with TACC1. We are using an *in vivo* system, the yeast two-hybrid assay for detecting potential protein-protein interaction by a functional complementation assay in yeast. Functional interactions between the target protein and proteins expressed in frame from the cDNA library of interest are detected by expression of two reporter genes in the yeast genome. Positive clones can then be isolated and sequenced. Coimmunopreciptitation and *in vitro* association techniques are then used to confirm that these interactions occur within mammalian cells.

Our initial search of potential TACC1 interacting proteins used an adult cDNA library, derived from bone marrow. This screen identified two known genes, SIAH1 and GAS41, two proteins implicated in potential growth control pathways in different cell types. Both of these proteins are expressed in the normal breast tissue. However, because antibodies are not currently available for SIAH1 and GAS41, we have fused these cDNAs to the hemagglutinin and EGFP epitopes. In *in vitro* association studies, we have shown that both recombinant GAS41, and SIAH1 interact with *in vitro* translated, radioactively labeled TACC1. These same constructs will now be introduced into the TACC1 expressing cells described above. The resulting HA-tagged protein should then

coimmunoprecipitate with TACC1 using anti-HA or anti-EGFP antibodies. We have determined that EGFP tagged GAS41 shows the same cellular distribution as TACC1. In addition, SIAH1 is localized to both the cytoplasm and the nucleus. The interactions between TACC1, SIAH1 and GAS41 could therefore occur in the cytoplasm and/or the nucleus. Hence we will also prepare extracts from different subcellular compartments, to determine whether the subcellular interaction between TACC1 and these factors is regulated. Because we have shown that overexpression of TACC1 causes aberrant accumulation of TACC1 in the cytoplasm and a potential disruption of the microtubule network, such interactions could also be altered in breast tumor tissues.

Recently a commercial mammary gland cDNA library (Clontech) has become available which has allowed us to identify TACC1 binding proteins in normal breast tissue. Our initial screen of one million cDNA clones from this library identified sixty-nine clones by nutrient selection. Encouragingly, a proportion of clones represented the SIAH1 and GAS41 proteins described above, suggesting that these proteins do interact with TACC1 in both normal breast tissue, and potentially in breast tumors. The remaining clones are currently being isolated and sequenced. The first series of clones isolated correspond to two recently identified proteins. The first protein identified, is the human orthologue of p16, a bovine protein involved in transport of proteins from the endoplasmic reticulum to the golgi apparatus prior to sorting to different subcellular compartments. The second protein identified, L-Sm7 is a component of a protein complex assembled in the cytoplasm and transported to the nucleus. This complex is a key component in splicing of pre-mRNAs. We have mapped the precise location of the binding site in TACC1 for these proteins by using smaller sections of the TACC1 cDNA and carrying out yeast two-hybrid analysis. The binding site for p16, and LSm-7 partially overlap those of GAS41 and SIAH1, suggesting that these proteins could compete with each other to bind TACC1. However, p16 and LSm-7 also bind to the conserved coiled coil domain, suggesting that these proteins could also interact with the TACC domain of TACC2 and TACC3. Antibodies to p16 and LSm-7 do not exist, therefore, we are currently cloning these cDNAs into vectors, which will allow expression of these proteins as EGFP fusion products. When transfected into mammalian cells, we will then be able to verify the interaction between TACC1 and these proteins by coimmunoprecipitation. Furthermore, we will assess whether these proteins interact with the other members of the TACC family. We will then be able to examine how these interactions alter during progression of breast tumors to higher grade metastatic carcinomas.

Interaction between TACC1 and GAS41 has been verified by coimmunoprecipitation. GAS41 has recently been shown to bind to the nuclear matrix protein NuMA. This protein is critical to the formation of the mitotic spindle. We have also shown that TACC1 binds weakly to the mitotic spindle during cellular division (Gergely et al 2000), but have been unable to demonstrate a direct interaction between NuMA and TACC1 by coimmunoprecipitation. One possibility is that GAS41 may regulate the interaction of TACC1 with another component of the mitotic spindle. The

functional significance of these interactions is currently unclear, however, sequence analysis has suggested that GAS41 is a potential transcription factor. This could implicate TACC1 in gene transcription. Verification of interactions with the other proteins are still ongoing. Our original task 6 was to screen cDNA libraries to identify full length genes intereacting with TACC1. This task has largely been unnecessary to this point since all of the genes showing interactions are available as full length sequences.

Task 1	Complete
Task 2	In Progress
Task 3	Complete
Task 4	Complete
Task 5	In progress
Task 6	Redundant

Key Research Accomplishments

1) Generation of a polyclonal antibody against TACC1 and the demonstration of expression of the protein in breast cancer cells.
2) Demonstration that TACC1 interactions with the cell cycle regulator gene GAS41 using immunoprecipitation.
3) Identification of the potential interaction between TACC1 and NuMa, a protein involved in the maintenance of the extracellular matrix.

Reportable Outcomes

Gergley F, Karlsson C, Still I, **Cowell JK**, Kilmartin J, Raff J. (1999) The TACC domin identifies a family of centrosomal proteins that can interact with microtubules. Proc Natl Acad Sci 97; 14352-14357.

Conclusions

We have generated a TACC1-specific polyclonal antibody which has allowed us to confirm that TACC1 interacts with GAS41 strongly suggesting a role in the control of the cell cycle in breast cancer cells. This view is further supported by the immunohistochemical demonstration of attachment of TACC1 to the mitotic spindle and potential interaction with the nuclear matrix protein NuMa. Attempts are underway to create stable, TACC1 overexpressing cell lines in order to further characterize the pathways involved in the transport of the TACC1 protein from the cytoplasm to the nucleus and how this promotes the malignant phenotype.